

CLAIMS

What is claimed is:

- 5 1. A method for screening a plurality of phage-displayed antibodies for an ability to bind to a radiation-inducible neoantigen present on a cell, the method comprising:
 - (a) contacting the cell with a first solution, the first solution comprising the plurality of phage-displayed antibodies;
 - 10 (b) isolating a second solution, the second solution comprising those phage-displayed antibodies that do not bind to the cell;
 - (c) removing any phage-displayed antibodies bound to the cell;
 - (d) treating the cell with radiation, wherein the treating results in a radiation-inducible neoantigen being present on the cell;
 - (e) contacting the cell with the second solution; and
 - 15 (f) detecting binding of a phage-displayed antibody to the radiation-inducible neoantigen on the cell.
- 20 2. The method of claim 1, wherein the plurality of phage-displayed antibodies comprise a phage-displayed single chain variable fragment (scFv) library or a phage-displayed Fab library.
3. The method of claim 1, wherein the phage-displayed antibodies are humanized.
- 25 4. The method of claim 1, wherein the phage-displayed antibody is encoded by a nucleic acid encoding a single chain variable fragment (scFv) antibody having an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 20, 22, and 24, or by a nucleic acid sequence that is selected from the group consisting of SEQ ID NOs: 17, 19, 21, and 23.
- 30 5. The method of claim 1, wherein the phage-displayed antibody has an amino acid sequence that is selected from the group consisting of SEQ ID NOs: 18, 20, 22, and 24.

6. The method of claim 1, wherein the phage-displayed antibody further comprises an epitope tag.

5 7. The method of claim 6, wherein the epitope tag is selected from the group consisting of a c-myc tag and a histidine tag.

8. The method of claim 1, wherein the cell is selected from the group consisting of a tumor cell and a vascular endothelial cell.

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9. The method of claim 8, wherein the vascular endothelial cell is present within tumor microvasculature.

10. The method of claim 1, wherein the radiation-inducible neoantigen
15 is selected from the group consisting of P-selectin, E-selectin, Endoglin, $\alpha_{2b}\beta_3$ integrin, and $\alpha_v\beta_3$ integrin.

11. The method of claim 1, wherein the detecting is by a technique
selected from the group consisting of ELISA, BIACORE, Western blotting,
20 immunohistochemistry, fluorometric microvolume assay technology, mass spectroscopy, MALDI-MS, and MALDI-TOF.

12. A method of targeting a therapeutic agent to a target tissue, the method comprising:

- 25 (a) providing an immunoconjugate composition comprising a therapeutic agent and an antibody or antibody fragment, wherein the antibody or antibody fragment is capable of binding to a radiation-inducible neoantigen;
- (b) irradiating the target tissue to induce expression of the
30 radiation-inducible neoantigen in the target tissue; and
- (c) contacting the irradiated target tissue with the immunoconjugate composition under conditions sufficient for binding of the antibody or antibody fragment to the radiation-

inducible neoantigen, whereby the therapeutic agent is targeted to the target tissue.

13. The method of claim 12, wherein the target tissue is a tumor or
5 tumor vasculature.

14. The method of claim 12, wherein the target tissue is present within a subject.

10 15. The method of claim 14, wherein the subject is a mammal.

16. The method of claim 12, wherein the immunoconjugate composition is polyvalent.

15 17. The method of claim 12, wherein the immunoconjugate composition further comprises a detectable label.

18. The method of claim 17, wherein the detectable label is detectable
in vivo.
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19. The method of claim 18, wherein the detectable label comprises a label that can be detected using magnetic resonance imaging, scintigraphic imaging, monochromatic X-ray, ultrasound, or fluorescence.

25 20. The method of claim 19, wherein the label that can be detected using scintigraphic imaging comprises a radionuclide label.

21. The method of claim 20, wherein the radionuclide label comprises
¹³¹I or ^{99m}Tc.
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22. The method of claim 12, wherein the therapeutic agent is selected from the group consisting of a virus, a radionuclide, a cytotoxin, a therapeutic gene, and a chemotherapeutic agent.

23. The method of claim 12, wherein the antibody or antibody fragment is humanized.

5 24. The method of claim 12, wherein the antibody or antibody fragment is a single chain fragment variable (scFv) antibody or an Fab antibody.

10 25. A method for suppressing the growth of a tumor in a subject, the method comprising:

- (a) exposing the tumor to radiation, whereby a radiation-inducible neoantigen is expressed; and
- (b) administering to the subject bearing the tumor an effective amount of an immunoconjugate composition, the
15 immunoconjugate composition comprising a therapeutic agent and an antibody or antibody fragment that binds to a radiation-inducible neoantigen, whereby growth of the tumor is suppressed.

20 26. The method of claim 25, wherein the antibody or antibody fragment is a single chain fragment variable (scFv) antibody or an Fab antibody.

25 27. The method of claim 25, wherein the tumor is selected from the group consisting of benign intracranial meningiomas, arteriovenous malformation, angioma, macular degeneration, melanoma, adenocarcinoma, malignant glioma, prostatic carcinoma, kidney carcinoma, bladder carcinoma, pancreatic carcinoma, thyroid carcinoma, lung carcinoma, colon carcinoma, rectal carcinoma, brain carcinoma, liver carcinoma, breast
30 carcinoma, ovary carcinoma, solid tumors, solid tumor metastases, angiofibromas, retrolental fibroplasia, hemangiomas, Kaposi's sarcoma, head and neck carcinomas, and combinations thereof.

28. The method of claim 25, wherein the subject is a mammal.

29. The method of claim 25, wherein the immunoconjugate composition comprises a liposome or a nanoparticle.

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30. The method of claim 29, wherein the nanoparticle further comprises a fluorescent label.

31. The method of claim 25, wherein the immunoconjugate composition is polyvalent.

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32. The method of claim 31, wherein the immunoconjugate composition comprises a plurality of antibodies or antibody fragments that bind to different epitopes, the plurality of antibodies or antibody fragments being selected from the group consisting of single chain fragment variable (scFv) antibodies, Fab antibodies, and combinations thereof.

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33. The method of claim 32, wherein the plurality of antibodies or antibody fragments comprises at least one antibody or antibody fragment that binds to an antigen present on a tumor cell and at least one antibody or antibody fragment that binds to an antigen present on a vascular endothelial cell.

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34. A single chain fragment variable (scFv) antibody isolated by the method of claim 1.

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35. An Fab antibody isolated by the method of claim 1.

36. An immunoconjugate composition comprising a single chain fragment variable (scFv) antibody or an Fab antibody that binds to a radiation-inducible neoantigen.

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37. The immunoconjugate composition of claim 36, wherein the immunoconjugate is polyvalent.

5 38. The immunoconjugate composition of claim 36, further comprising a therapeutic agent.

39. The immunoconjugate composition of claim 38, wherein the therapeutic agent is selected from the group consisting of a virus, a radionuclide, a cytotoxin, a therapeutic gene, and a chemotherapeutic agent.
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40. The immunoconjugate composition of claim 36, wherein the single chain fragment variable (scFv) antibody or Fab antibody is humanized.

41. The immunoconjugate composition of claim 36, wherein the single chain fragment variable (scFv) antibody is encoded by a nucleic acid encoding an scFv antibody having an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 20, 22, and 24, or by a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 17, 19, 21, and 23.
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42. The immunoconjugate composition of claim 36, wherein the single chain fragment variable (scFv) antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 20, 22, and 24.

25 43. The immunoconjugate composition of claim 36, in a pharmaceutically acceptable carrier.

44. The immunoconjugate composition of claim 36, further comprising a detectable label.
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45. The immunoconjugate composition of claim 44, wherein the detectable label is detectable *in vivo*.

46. The immunoconjugate composition of claim 45, wherein the detectable label comprises a label that can be detected using magnetic resonance imaging, scintigraphic imaging, ultrasound, or fluorescence.

5 47. The immunoconjugate composition of claim 46, wherein the label that can be detected using scintigraphic imaging comprises a radionuclide label.

10 48. The immunoconjugate composition of claim 47, wherein the radionuclide label comprises ^{131}I or $^{99\text{m}}\text{Tc}$.

15 49. A polyvalent immunoconjugate composition, the polyvalent immunoconjugate composition comprising a plurality of antibodies or antibody fragments selected from the group consisting of single chain fragment variable (scFv) antibodies, Fab antibodies, and combinations thereof, wherein the plurality of antibodies or antibody fragments bind to a plurality of different epitopes, and wherein at least one of the epitopes is present on a radiation-inducible neoantigen.

20 50. The polyvalent immunoconjugate composition of claim 49, wherein at least one of the plurality of different epitopes is present on a vascular endothelial cell.

25 51. A method for prioritizing the binding of a plurality of antibodies or antibody fragments to a target tissue in a subject, the method comprising:

30 (a) providing a plurality of antibodies or antibody fragments that bind to the target, wherein the plurality of antibodies or antibody fragments comprise at least two different antibodies or antibody fragments that bind a radiation-inducible neoantigen within the target tissue, and wherein the at least two different antibodies or antibody fragments are distinguishable from each other;

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- (b) irradiating the target tissue, whereby the radiation-inducible neoantigens are expressed within the target tissue;
- (c) administering the plurality of antibodies or antibody fragments to the subject under conditions sufficient to allow the plurality of antibodies or antibody fragments to bind to the radiation-inducible neoantigen in the target tissue;
- (d) isolating a portion of the target tissue from the subject, wherein the portion comprises the radiation-inducible neoantigens to which the plurality of antibodies or antibody fragments bind;
- 10 (e) identifying the at least two different antibodies or antibody fragments in the portion of the target tissue;
- (f) comparing a relative selectivity and an affinity for the radiation-inducible neoantigens of the at least two different antibodies or antibody fragments identified in step (e) in the irradiated target tissue; and
- 15 (g) assigning a priority to the at least two different antibodies or antibody fragments based on the comparing of step (f).

20 52. The method of claim 51, wherein the subject is a mammal.

53. The method of claim 51, wherein the target tissue is a tumor or tumor vasculature.

25 54. The method of claim 51, wherein the antibodies or antibody fragments are single chain fragment variable (scFv) antibodies, Fab antibodies, or combinations thereof.

55. The method of claim 54, wherein the antibodies or antibody fragments are humanized.

30 56. The method of claim 51, wherein the at least two different antibodies or antibody fragments that bind to at least two different radiation-

inducible neoantigens are distinguishable from each other based upon differences in molecular weight.

57. The method of claim 51, wherein the at least two different
5 antibodies or antibody fragments that bind to at least two different radiation-inducible neoantigens within the target tissue each further comprises a different detectable label, such that the antibodies or antibody fragments that bind to different radiation-inducible neoantigens can be distinguished from each other.

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58. The method of claim 57, wherein the different detectable labels are fluorescent labels, and each fluorescent label has a different excitation or emission spectrum, such that the different antibodies can be distinguished from each other.

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59. The method of claim 51, wherein the at least two radiation-inducible neoantigens within the target tissue are selected from the group consisting of P-selectin, E-selectin, Endoglin, $\alpha_2\beta_3$ integrin, and $\alpha_v\beta_3$ integrin.

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60. The method of claim 51, wherein the administering is by intravenous injection or intratumoral injection.

61. The method of claim 51, wherein the portion is a tumor biopsy.

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62. The method of claim 51, wherein the detecting is by mass spectroscopy.

63. A method of detecting a tumor in a subject, the method
30 comprising:

- (a) exposing a suspected tumor to ionizing radiation;
- (b) administering to the subject an immunoconjugate composition, wherein the immunoconjugate composition comprises an

antibody or antibody fragment that binds to a radiation-inducible neoantigen and a detectable label; and

(c) detecting the detectable label, whereby a tumor is diagnosed.

5 64. The method of claim 52, wherein the antibody or antibody fragment is a single chain fragment variable (scFv) antibody or an Fab antibody.

10 65.A method for detecting a tumor in a subject, the method comprising:

(a) exposing a suspected tumor to ionizing radiation;

(b) removing a portion of the suspected tumor;

15 (c) contacting an immunoconjugate composition with the suspected tumor *in vitro*, wherein the immunoconjugate composition comprises an antibody or antibody fragment that binds to a radiation-inducible neoantigen and a detectable label; and

(d) detecting the detectable label, whereby a tumor is diagnosed.

20 66. The method of claim 65, wherein the antibody or antibody fragment is a single chain fragment variable (scFv) antibody or an Fab antibody.